FABRICATION OF NOVEL PAPER-BASED BIOSENSOR FOR DIAGNOSIS OF DROUGHT STRESS IN PLANTS

Min Kyu Im[1], Mi Rha Lee[2], Sumaiya Islam[1], Young-Soo Choi[2*], Kyeong-Hwan Lee [1*],

[1]*Department of Rural and Biosystems Engineering, Chonnam National University, Gwangju, 61186, Korea,*
[2]*Agricultural Robotics and Automation Research Center, Chonnam National University, Gwangju, 61186, Korea*

[*]*Corresponding author: Young-Soo Choi(memschoi@gmail.com), Kyeong-Hwan Lee(khlee@jnu.ac.kr)*

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INTRODUCTION

❖ Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world (Passioura, J.B., 1996, 2007). Therefore early diagnosis of crop drought stress is an essential part of minimizing crop loss and appropriate management.

❖ Proline is an osmotic agent that plays a role in the growth of crops against environmental stress especially drought (Hare and cress., 2007). By ninhydrin assay (Ruhemenn's purple) proline content can be measured (Bates et al., 1973).

❖ Recently, the paper-based analytical device has been suggested as an alternative, complement, or improvement to point-of-care testing area (Parolo., 2013; Yestisen , A.K., 2013).

❖ The objective of this study was to develop a fully enclosed microfluidic paper-based sensors for diagnosing drought stress without contamination from the outside environment.

MATERIAL & METHODS

❖ Schematic of paper based biosensor

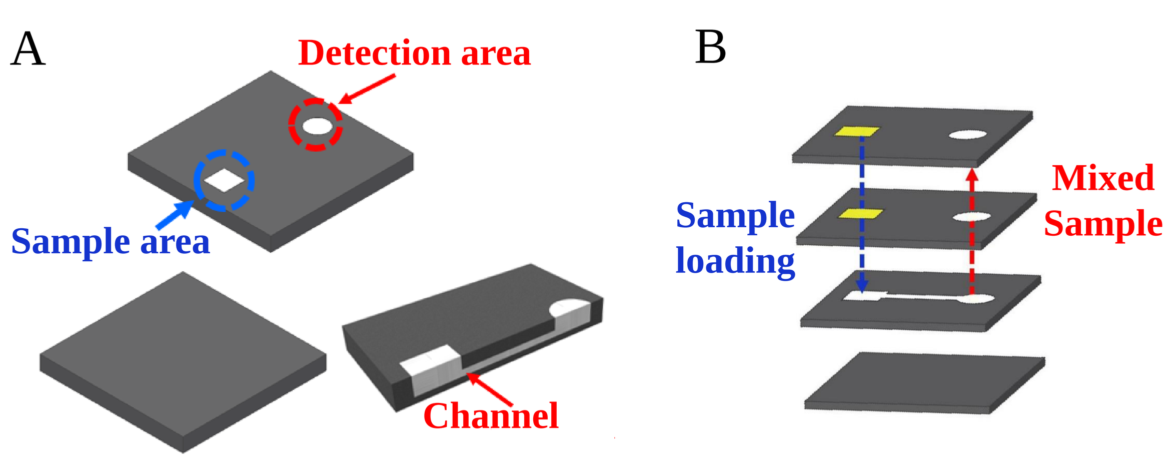

Figure 1. Schematic illustration of paper based biosensor. (A) Top view and cross-sectional views of wax patterned paper sensor. (B) operating principle of sensor.

❖ Principle of diagnosis drought stress

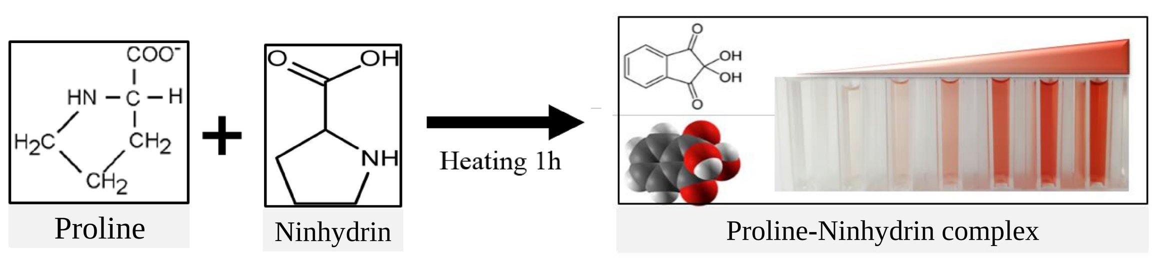

Figure 2. Measuring principle of proline which is proportional to drought stress.

❖ Reagents

Final concentration	1mM Proline	Slfosalycilic acid
50μM	50μl	950μl
100μM	100μl	900μl
200μM	200μl	800μl
300μM	300μl	700μl
400μM	400μl	600μl

- 1% sulfosalicylic acid
- Ninhydrin reagent mixture
- Pure proline powder

❖ Characteristics of paper based sensor

Paper substrate	Wax printer	Sensor size
Whatman chromatography No. 1 paper	Xerox, ColorQube 8570DN	Sample area – 8mm x 8mm Detection area - Ø 6mm

❖ Data acquisition and analysis

Heating for various temperatures and times, and images of the detection part were analyzed with RGB color intensity program.

❖ Fabrication or paper-based microfluidic sensor

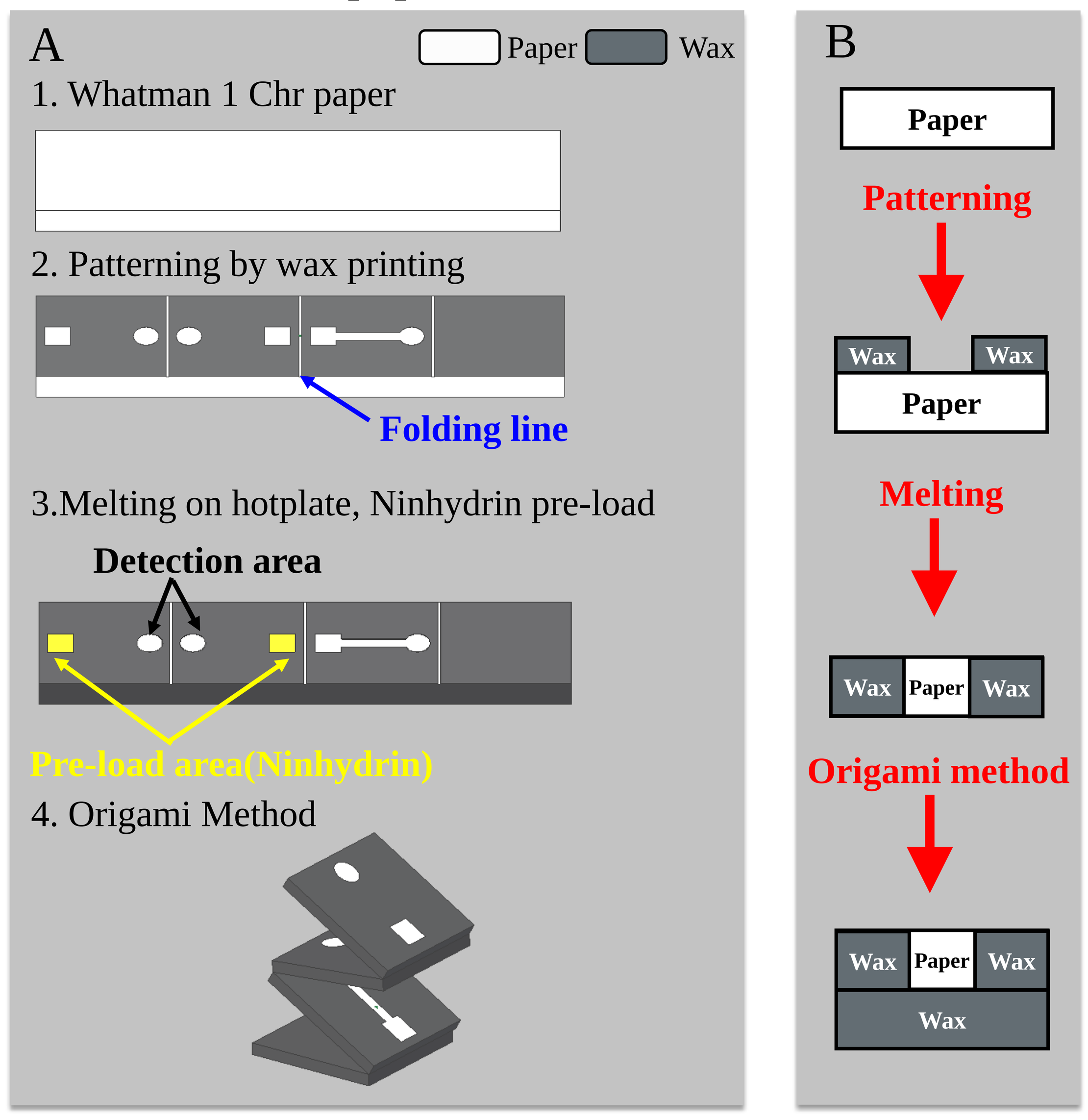

Figure 3. Schematic illustration of fabrication process by wax printing. (A) Top view and (B) Cross-sectional views of wax patterned paper sensor.

RESULTS & DISCUSSION

❖ Optimization of wax impregnation into the paper

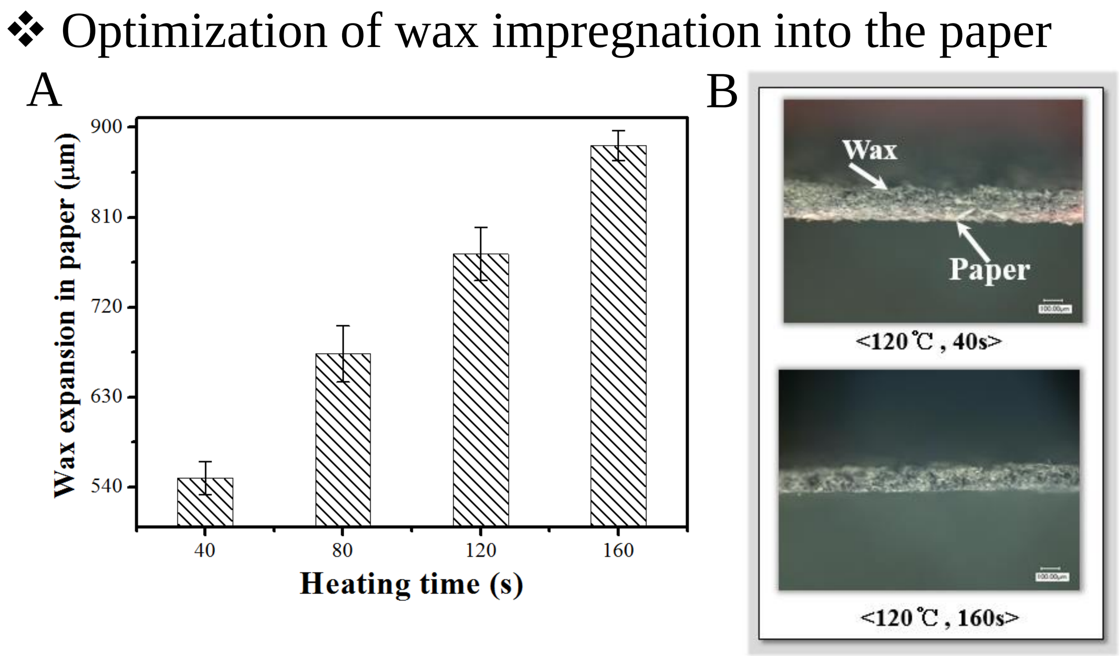

Figure 4. Determination of heating of wax printing. (A) The degree of the wax that melts into the paper according to heating time. (B) Cross-sectional view of wax expansion in paper.

❖ Optimization of sensor design

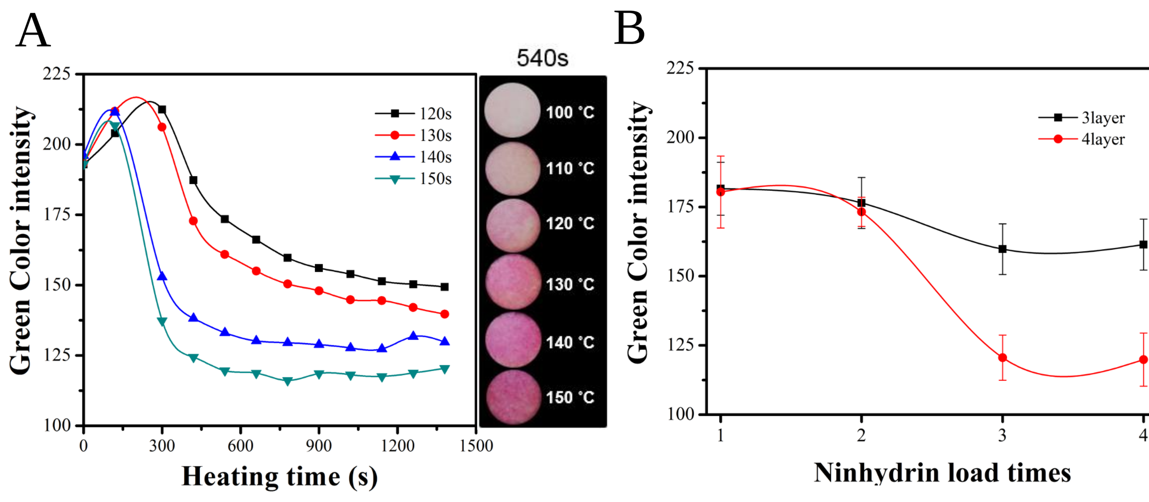

Figure 5. (A) Green color intensity at heating temperature and time to obtain optimized ninhydrin-proline reaction in fabricated paper sensor. (B) Green color intensity according to ninhydrin pre-load amount.

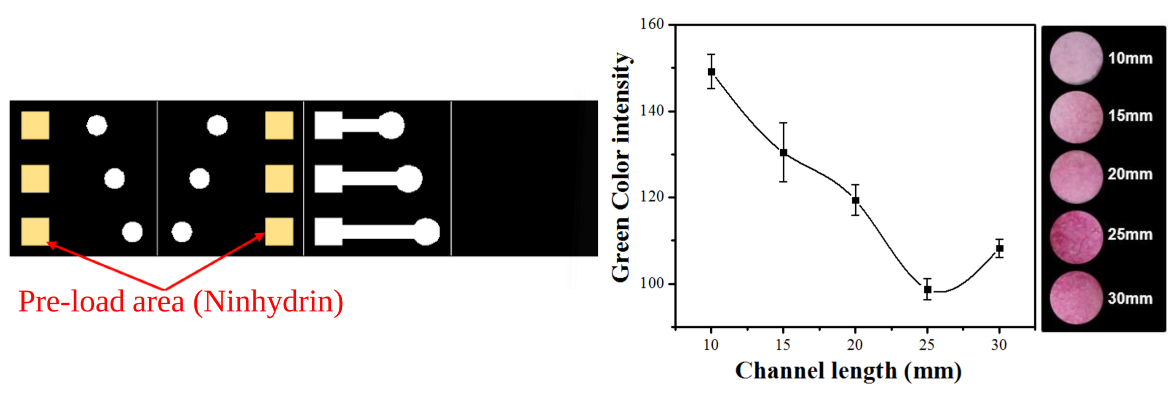

Figure 6. Optimization of the channel length where the reaction of pre-loaded ninhydrin solution and sample occurs.

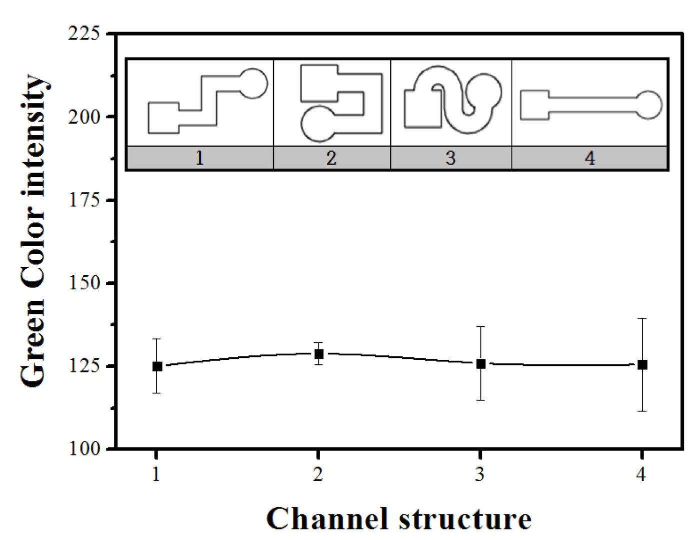

Figure 7. Green color intensity according to the channel structure, when channel length is fixed.

❖ Measurement range of paper sensor using chemical proline

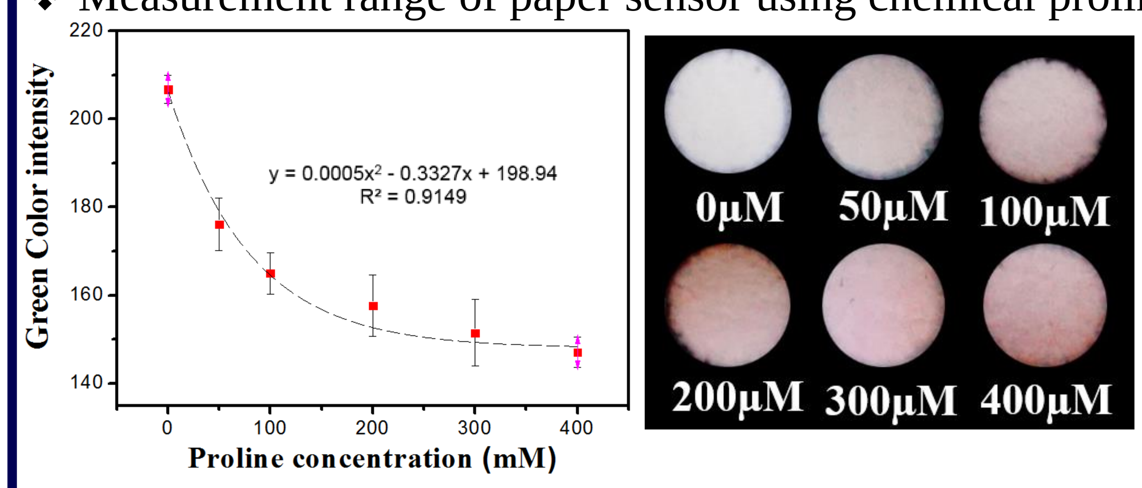

Figure 8. Diagnosis of pure chemical proline reagent in optimized sensor.

❖ Drought stress diagnosis with *Arabidopsis*

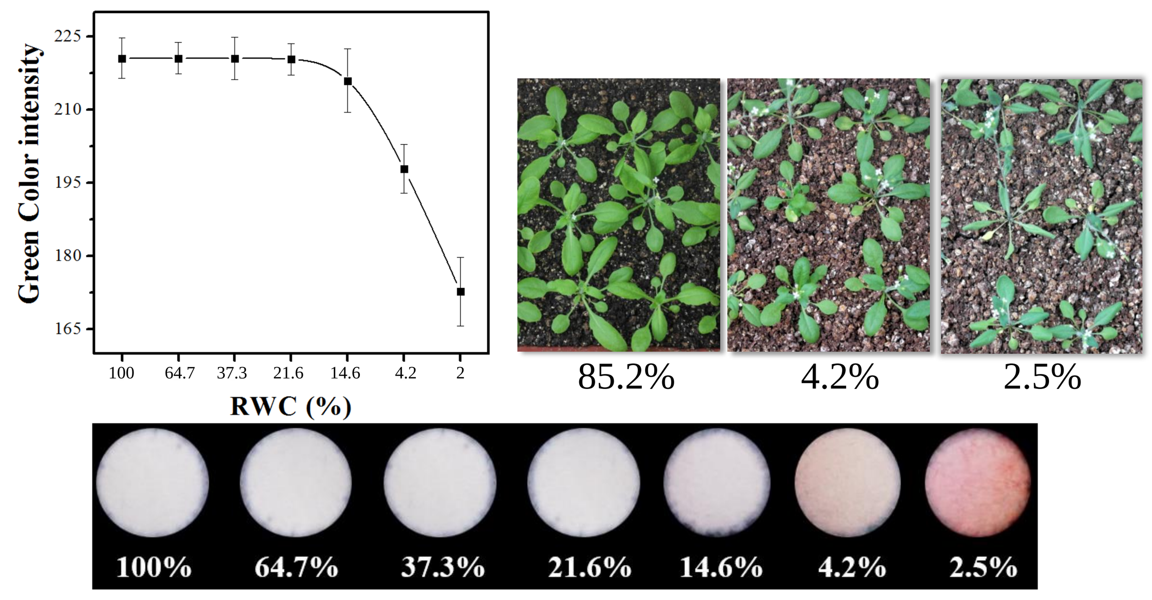

Figure 9. Diagnosis drought stress of *Arabidopsis* in optimized sensor. (A) Green Color intensity according to RWC. (B) The *Arabidopsis* state according to each drought stress. (C) Analysis results from paper sensor.

❖ Proline concentration of *Arabidopsis* using spectrophotometer

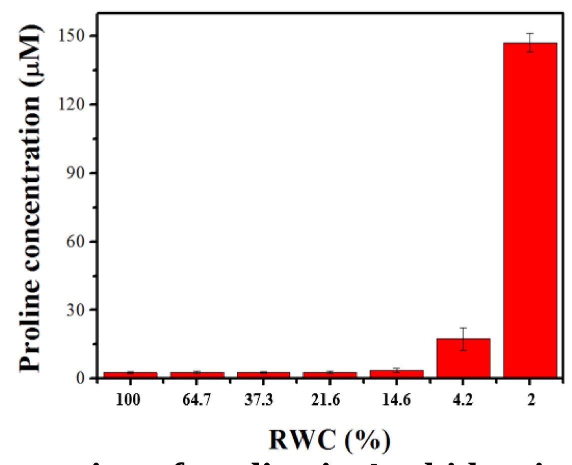

Figure 10. Concentration of proline in Arabidopsis according to RWC.

CONCLUSIONS

❖ We describe the fabrication of paper-based fully enclosed microfluidic sensor that can diagnose plant drought stress in an early stage.

❖ The Ninhydrin reaction on the paper sensor was optimized at 150˚C for 540s.

❖ We were able to detect the proline, a biomarker for plant drought stress, using a paper-based fully enclosed microfluidic sensor.

❖ We expect that this bioactive paper-based sensor has great potential for diagnosis of drought stress of crop.

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